PAX3

Cis-Regulatory Elements

Transgenic mouse analysis demonstrated that **the 1.6 kb putative promoter region upstream of *Pax3* directs gene expression in the dorsal neural tube**, but is not sufficient to direct somite or craniofacial expression (Li et al., 1999, Natoli et al., 1997). Deletion analyses suggested two distinct regions within the 1.6kb promoter were necessary for *Pax3* expression in the neural tube (Natoli et al., 1997).

Four sub-regions contained within the previously identified two regions of the -1.6 kb *Pax3* promoter were identified by electrophoretic mobility assays using promoter deletion constructs expressed in P19 embryonic carcinoma cells stimulated with retinoic acid. **The proteins identified that bound to these four sub-regions were BRN1, BRN2, and members of the PBX and MEIS families, with evidence that PBX members complex with HOX family members at two of these sites, thus demonstrating *Pax3* is regulated by both neural and anterior-posterior transcription factors**. Specifically, BRN2 together with HOXA1 showed significant activation of the *Pax3* promoter. Analysis of transgenic mice carrying *Pax3* promoter- gal constructs harboring mutations in the four sub-regions suggested that two of the binding sites function as transcriptional activators, and the other two as repressors (Pruitt et al., 2004).

Further analysis of the 1.6 kb region upstream of *Pax3* identified two highly conserved regions, named NCE1 and NCE2. NCE1 overlapped completely with one of the regions previously identified by (Natoli et al., 1997). **Deletion of either of these regions abolished dorsal neural tube-directed expression** in -gal transgenics and a minimal promoter construct containing both of these regions was sufficient to direct -gal expression in the neural tube and neural crest derivatives. TEAD2, complexed with YAP65, binds directly to NCE2 of the *Pax3* promoter. In situ hybridization showed co-localization of *Pax3*, *Tead2*, and *Yap65* in dorsal neural tube. A variety of assays showed that wild type **TEAD2 activates *Pax3* transcription by binding to NCE2**. Of note, the minimal promoter region containing NCE1 and NCE2 does not fully recapitulate normal *Pax3* expression, as cervical and caudal regions showed absent or ectopic expression, and neural tube expression was more dorsally restricted (Milewski et al., 2004).

In B16F1 mouse melanoma cells, the -1.6 kb *Pax3* promoter region was shown insufficient to activate transcription, while the larger -14 kb region sufficiently activated transcription. However, mutation of the four previously identified binding regions within the -1.6 kb region affected *Pax3* transcription, suggesting these binding elements still regulate transcription, but other upstream elements are also important for *Pax3* expression in melanocytes. Comparative sequence analysis between mouse and human identified a 1.1 kb region of conserved sequence in the *Pax3* promoter at -6.9 to -5.8, and the authors suggest additional *Pax3* transcriptional regulation occurs via this region. OCT1 and BRN2 binding was confirmed at two of the previously described binding sites within the -1.6 kb region of the *Pax3* promoter, with both activating transcription. PBX and PREP1 were shown to complex together at the remaining two sites, where they repressed *Pax3* expression (Zhu and Pruitt, 2005).

Transgenic mice expressing Cre recombinase under control of the 1.6 kb *Pax3* promoter directed Cre expression to neural crest tissues, but not somites, in a pattern similar to that of endogenous *Pax3*. These mice can facilitate specific gene deletions in *Pax3*-expressing neural crest cells (Li et al., 2000).

Construction of a transgenic mouse line, *Tg(Pax3-tv-a)HPvn*, in which the avian RCAS vector TVA is expressed under control of the 1.6kb *Pax3* promoter, demonstrated that this construct allowed targeting of genes to the melanocyte lineage via RCAS infection of neural crest cultures. This transgenic can be used to test the roles of various genes in melanocyte development (Hou et al., 2004).

Comparative genomic sequence analysis of the -15 to -6 kb region upstream of *Pax3* in human and mouse identified 3 highly conserved regions. Subsequent creation of a panel of transgenic mice harboring

B-gal under control of various combinations of these regions showed the **presence of a 291-bp element (located at approximately -6kb) that specifically directed *Pax3* expression in muscle precursor cells** that arise from the ventrolateral lip of the somite and migrate to the limbs, tongue, and skeletal muscle. This expression began at E9.0, later in development than initial *Pax3* expression elsewhere in the embryo, and appeared independent of *Pax3* function, as -gal expression in hypaxial muscle was still seen in transgenic mice on a Splotch background (Brown et al., 2005).

Regulatory sequences located at positions -135 to -98 in human *PAX3* are directly bound and regulated by a SMAD4-SKI complex. This complex becomes activated by TGF-beta signaling that originates from keratinocytes, resulting in inhibition of *Pax3* mRNA transcription in melanocytes. These regulatory sequences are conserved across human, monkey, rat, and mouse (Yang et al., 2008).

Targeted deletion of a 674bp region upstream of the mouse *Pax3* locus (named NCE) which contains previously identified upstream enhancer regions (see Milewski et al., 2004) did not impair *Pax3* expression in neural crest, suggesting the NCE was not solely responsible for *Pax3* expression. Of note, the mice retaining the floxed PGK-neomycin cassette showed disrupted *Pax3* expression, exhibiting phenotypes identical to those of Pax3^{Splotch} mice. Crossing of these mice retaining the floxed cassette with neural crest-specific or hypaxial somite-specific CRE constructs allowed tissue-specific rescue of *Pax3* expression, and complete phenotypic rescue of the CRE-expressing tissue types in these mice suggested that the PAX3-dependent developmental pathways in neural crest and somites do not regulate one another. Analysis of intronic and downstream evolutionarily conserved regions at the *Pax3* locus identified **an enhancer located within the 4th intron (named ECR2), which, along with a minimal *Pax3* promoter, could direct reporter gene expression even in the absence of the NCE**. ECR2 also directed neural crest expression of a reporter construct in transient transgenic zebrafish, and this expression required functional Lef/TCF sites within ECR2 (Degenhardt et al., 2010).